Saskatchewan

A D F

Agriculture Development Fund

FINAL REPORT

19980291

EFFECTS OF OZONE TREATMENTS ON SHELF-LIFE EXTENSION, PHYSIOLOGY AND QUALITY OF FRESH SASKATOON BERRIES

Funded by
The Agriculture Development Fund

March 2002
Prepared by: University of Saskatchewan

SAF
Saskatchewan Agriculture & Food

Adding value to agriculture...through research and development

102-04989/

Effects Of Ozone Treatments On Shelf-life Extension, Physiology And Quality Of Fresh Saskatoon Fruit

ADF Project #98000291

Final Report - February 15, 2002

Annette Zatylny & Richard St-Pierre
Native Fruit Development Program
Department of Plant Sciences
University of Saskatchewan
Saskatoon

1. Abstract

This project determined that ozone, used as a surface disinfectant on saskatoon fruit, was effective in decreasing microbial load without compromising fruit quality, thus helping to extend fruit shelf life. Exposure to ozone at the rate of 0.8 ppm for one to two hours prior to the packaging of saskatoon fruit was effective in reducing total microbial populations on fruit by 14%. Yeast populations were reduced by 10 - 20% and mold populations were reduced by up to 20%. Fruit quality was little or not affected by ozone treatment. Further research aimed at increasing the effectiveness of ozone and at examining the economics of its use would be useful.

2. Executive Summary

Increased production of saskatoon fruit in Saskatchewan has prompted concerns about post-harvest storage practices. Due to rapid deterioration in fruit quality, freezing immediately after harvest is critical. If a fresh-pack industry needs to be developed with potential for an export market, robust measures have to be developed to maintain the quality of fresh fruit for a considerable period of time.

Extending the shelf-life of horticultural commodities can be accomplished by retarding the metabolic processes associated with quality loss (usually achieved by lowering storage temperature), or by controlling pathogenic activity using post-harvest treatments such as ozone. Ozone is a natural product. It acts as a surface disinfecting agent to reduce microbial loads as demonstrated in blackberries and grapes, and as an ethylene absorber, controlling the amounts of ethylene in a storage environment. Because saskatoon fruit are climacteric (i.e. ethylene controls

ripening), methods to retard ethylene production would enhance shelf-life.

Preliminary results indicated that a single application of ozone injected into bags of fruit just prior to storage was not effective in reducing microbial populations on saskatoon fruit. The half-life of ozone is 15-50 minutes. Therefore, it was possible that ozone had disassociated into oxygen before its effect as a surface sterilizing agent was established. Consequently, modifications were made to experimental protocols to assess the effects of a continuous application of ozone for a given length of time on saskatoon shelf-life and fruit quality.

In 2000, saskatoon fruit of the cultivars Pembina, Honeywood, Thiessen, and Smoky were exposed to various concentrations (0 - control, 300 mg/h, and 600 mg/h) of ozone for two hours in a ventilated room. The latter two concentrations were measured as 0.4 ppm and 0.8 ppm by an ozone meter; 600 mg/h represented the highest level of output by the ozone generator. The temperature of the room was 0°C; this temperature is the optimal storage temperature for saskatoon fruit. Following the ozone treatment, fruit were packaged in modified atmosphere (MA) bags, heat-sealed and stored at 0°C for 28 days. These bags have been shown to be effective in prolonging the shelf-life of saskatoon fruit.

In 2001, saskatoon fruit of the cultivars of Thiessen, Honeywood and Smoky were exposed to ozone for one hour in a 0°C cooler. Two ozone generators set at 300 mg/h were placed underneath trays of fruit (equivalent to 0.8 ppm). Spore suspensions of *Botrytis* and *Alternaria* were sprayed on the fruit. Two controls, sprays with distilled water and unsprayed fruit, also were used. Fruit were allowed to dry at room temperature for two hours after spray treatment and prior to ozone exposure. After ozone treatment, fruit were bagged in polyethylene freezer bags and stored at 0°C for 3 weeks.

Fruit quality and microorganism content were assessed at harvest and each subsequent week during storage. Soluble solids content, pH, titratable acidity (in 2001 only), Hunter L value, hue angle, texture and yeast and mold populations were used as indices of fruit quality. Soluble solids content and pH are indicative of fruit flavour; high soluble solids content and low pH are associated with increased sugars and increased acidity, respectively. Hunter L value and hue angle are indicative of fruit colour. A low L value is associated with darker fruit and a high hue angle is indicative of a reddish hue. A high value for fruit texture indicates firmer fruit.

Exposure to ozone at the rate of 0.8 ppm for one to two hours prior to the packaging of saskatoon fruit was effective in reducing total microbial populations on the fruit by 14% in both years of the study. Yeast populations of ozone-treated fruit were significantly reduced by 20% in 2000 and 10% in 2001 although this latter decrease was not significant. In 2001, mold populations were significantly reduced by 20% due to ozone treatment. The modified atmosphere bags used in 2000 maintained very low microbial populations during storage compared to the polyethylene bags and probably masked some of the effectiveness of the ozone treatment. Mold populations were apparently more affected than yeast by the MA bags, decreasing during storage.

In 2000, fruit pH, Hunter L value and hue angle were increased due to ozone exposure by 1%, 4% and 3%, respectively. In 2001, fruit pH was reduced by 1% and hue angle was increased by 2% due to ozone exposure otherwise no significant change occurred in fruit quality. It is not known why pH was increased during the first year of the experiment and decreased in the second. Overall, these small changes to fruit pH and colour (slightly lighter and more reddish fruit), although statistically significant, do not represent a reduction in fruit quality for all practical purposes.

In conclusion, ozone treatment was effective in decreasing the surface microbial content of saskatoon fruit without compromising fruit quality. Further research aimed at increasing the effectiveness of ozone and examining the economics of its use would be useful.

3. Technical Report

3.1. Background

The need to develop a fresh market industry for saskatoon fruit in Saskatchewan requires robust methods for storage. On-going research on saskatoon fruit has demonstrated that under controlled conditions of temperature and by restricting respiratory activity, the shelf life of fresh fruit can be prolonged for a considerable period of time. However, with concerns about the microbial safety of fresh produce (Garrett and McInerney, 1997) it is becoming increasingly important to comply with regulatory guidelines on acceptable levels of microbes in fresh produce. Ozone has been reported to be effective in reducing microbial loads thereby extending the shelf life of blackberries and grapes (Barth *et al.*, 1995; Sarig *et al.*, 1996). Levels as low as 0.3 ppm were reported to be effective for blackberries (Barth *et al.*, 1995).

The objectives of this project were: (1) to investigate the effects of various levels of ozone on shelf-life extension of fresh saskatoon fruit stored under modified atmosphere conditions; and (2) to examine the effects of ozone on fruit quality and physiology.

3.2. Project Trials - 1998

3.2.1. Materials and Methods

3.2.1.1. Preliminary Activities

A basic requirement of this project was to inject ozone (O_3) into plastic bags containing fresh saskatoon fruit. Because O_3 is highly unstable (half-life of 15-50 min) it was necessary to generate it on-site. Ozone generators (Crystalair, Delta, BC)(Figure 3.2.1.1.) were comprised of an ozone level dial (to control the O_3 concentration), a fan speed dial (to control airflow), and an inlet and outlet port. Ozone was generated by exposing air to an electric current which splits O_2

to form O_3. The O_3 concentration (ppm) in these generators was controlled by either adjusting the amount of air or adjusting the current.

A number of modifications were made to the generator. (1) The diameter of the outlet port was decreased from 3.75 cm to 0.16 cm. This reduction was necessary to produce a steady stream of gas that could be used to fill up the bags. Fittings were obtained from Saskatoon Valve and Fitting (Saskatoon, SK). All materials and fittings were inert or made of stainless steel because O_3 is highly reactive. (2) A hose was connected from the outlet port which contained a needle-like attachment. This would allow for flexibility when filling up bags. (3) A high-pressure rubber tubing (used for compressed air lines) was attached to a flow meter (model # CON 565-951100, Praxair, Saskatoon, SK) and subsequently to the generator. This was done to ensure a constant supply of air to the generator. (4) The generator was made leak-proof by sealing leak points with hot glue. Other sealants were found to react with O_3.

An ozone meter (model #AMC-1108, Armstrong Manufacturing Corp., Nepean, ON) (Figure 3.2.1.2.) was used to monitor O_3 concentrations by placing the hose from the outlet port of the generator as close as possible to the sensor of the meter. The O_3 level dial was adjusted to a given level, left to stabilize for about 3 minutes and concentrations were recorded. This procedure was repeated for several levels on the dial to establish a relationship between dial number and O_3 concentration. Results indicated that O_3 concentrations were within ± 1ppm.

3.2.1.2. Initial Trials On Fruit

Initial trials were made: (1) to establish a range of O_3 concentrations that could be used for saskatoon fruit; (2) to develop criteria for fruit quality assessment; and (3) to establish protocols for continuous application of O_3. These initial trials were conducted on cherries and strawberries so that the experimental protocol would be established by the time the saskatoons were ripening.

Cherries, relatively bruise-free, were placed in plastic bags and sealed using a vacuum packer (model # KVP-400A, Process Plus Inc., Phoenix, AZ) (Figure 3.2.1.3.). Each bag contained 10 cherries. Fruit were held at 0°C until required. A single application of O_3 was performed by piercing the bags with the needle-like attachment connected from the O_3 generator, injecting gas at a given concentration and sealing the hole immediately. The concentrations used were 0, 5, 10, 15, 20, 25, 30, 35 and 40 ppm. Fruit were stored at 0°C and 25°C for 5 days. Fruit quality was assessed subjectively. Three replicates were performed.

Because O_3 dissipates readily, the rationale for a continuous application was to compare its effectiveness to a single application. Strawfruit (harvested from the U of S plots) were put in cardboard trays and were placed in sealed tubs through which O_3 was continuously pumped for 2 hours. Fruit were stored at 25°C for 2 days. Fruit quality was assessed visually. No fruit were stored at 0°C as the rationale was to develop protocols.

3.2.1.3. Trials on Saskatoon Fruit

Fruit of the saskatoon cultivars, Smoky and Pembina, were mechanically harvested from the University of Saskatchewan (U of S) plots, Saskatoon. Care was taken to ensure maintenance of fruit quality after harvest. Fruit, relatively free of bruising and damage, were placed in modified atmosphere (MA) and freezer bags, vacuum-sealed and held at 0°C until required in order to compare the effectiveness of O_3 treatments in these bags. Fruit were exposed to single applications of O_3 at 0, 1 and 30 ppm and stored at 0°C and 25°C. Microbial quality was assessed at 0, 2, 4, 8, 16 and 32 days on whole fruit for internal fungal loads and on fruit washes for surface pathogen loads. Petrifilms (3M Canada, Inc., London, ON) for yeast and molds and for total aerobic count were used to plate dilutions from fruit washes. Total aerobic, and yeast and molds colonies were counted. Fruit quality was assessed subjectively.

For continuous application of O_3, plastic trays (with slots at top, bottom and sides) containing fruit were placed in perforated tubs and exposed to concentrations of 0, 3 and 6 ppm O_3 (Figure 3.2.1.4.). Tubs were stacked on top of each other and placed in a 15°C cooler. This cooler was selected because it was equipped with an inlet and outlet fan for ventilation. Ozone was applied continuously for 2 weeks and the cooler was vented each day for approximately 5 minutes. Fruit quality was assessed visually.

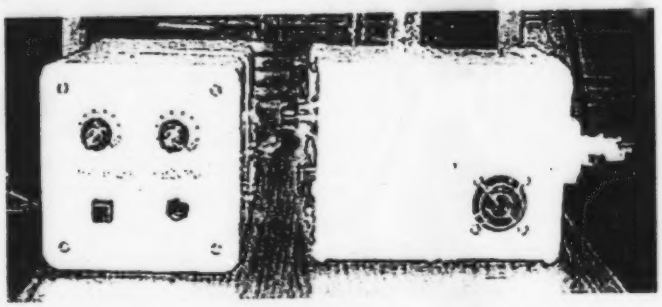

Figure 3.2.1.1. Ozone generator showing front and side views.

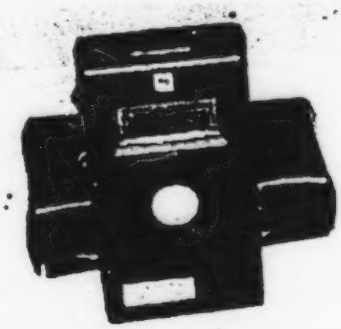

Figure 3.2.1.2. Ozone meter

Figure 3.2.1.3. Vacuum packer

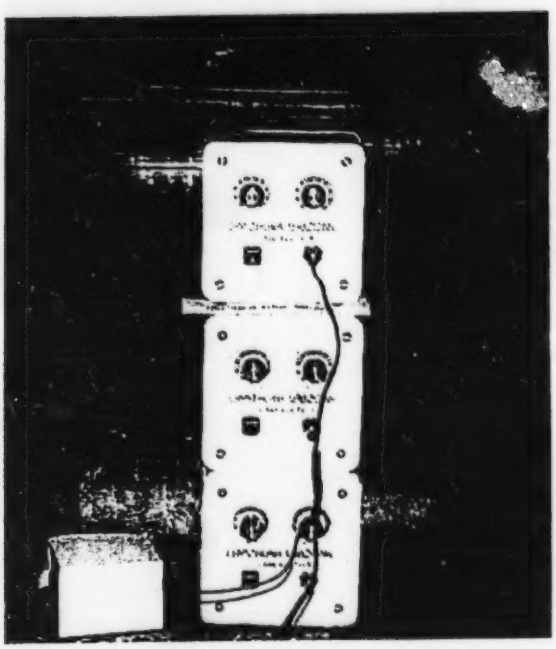

Figure 3.2.1.4. Experimental setup showing continuous application of ozone.

3.2.2. Results, Discussion and Conclusions

Fruit quality for the cherries that were exposed to various O_3 concentrations and stored at 25°C and 0°C for 5 days was rated subjectively. Microbial growth and poor fruit quality such as pitting and wrinkling (resulting from moisture loss) were the criteria for rating. The results showed that fruit stored at 0°C generally maintained better textural and colour quality than fruit stored at 25°C; microbial growth was also more apparent at 25°C than at 0°C. Decreasing the storage temperature retarded respiratory and other metabolic activities thereby maintaining quality and prolonging shelf life.

Because degradation in fruit quality was more apparent at 25°C, subjective ratings for fruit exposed to various O_3 concentrations were based on this storage temperature. If any of the above named disorders (fungal growth, pitting and wrinkling) was found in a given replicate of an O_3 treatment, that O_3 concentration was considered to be ineffective in maintaining fruit quality. Fruit exposed to 35 and 40 ppm showed signs of fungal growth whereas fruit exposed to 10, 15, 20 and 25 ppm appeared wrinkled and pitted, and also showed signs of fungal growth. Fruit exposed to 5 and 30 ppm were of moderate quality; generally appeared firm and of good colour with no obvious signs of fungal growth in any of the three replicates. These results were puzzling because it was expected that an increase in O_3 concentration would result in decreased microbial growth. Due to time constraints (with respect to the time frame for saskatoon harvest) the experiment was not repeated.

Results for the continuous application of O_3 on strawfruit showed excessive microbial growth after 2 days at 25°C. It was noted that the cardboard trays which contained fruit might have absorbed the gas. This finding stressed the need to store fruit in containers that would be unreactive to O_3.

For the experiment on saskatoon fruit, 5 and 30 ppm were selected as the O_3 concentrations. 30 ppm represented effective pathogen control with risk of physiological damage, whereas 5 ppm compromised pathogenic control for decreased risk of physiological breakdown. Because saskatoon fruit have higher respiratory rates than cherries and would be more susceptible to physiological breakdown, 1 ppm (rather than 5 ppm) was considered as the ideal O_3 concentration representing the lower limit which compromised pathogenic control for decreased risk of physiological breakdown. Subsequently, 1 and 30 ppm were used for the experiment on saskatoon fruit.

3.3. Project Trials - 1999

3.3.1. Materials and Methods

3.3.1.1. Harvest and Post-harvest Handling

Saskatoon fruit of the cultivars Smoky and Pembina were mechanically harvested from the U of S plots, Saskatoon. Fruit, relatively free of bruising and damage, were placed in modified atmosphere (MA) and freezer bags and sealed in order to compare the effectiveness of O_3 treatments in these bags. Fruit were exposed to single applications of O_3 at 0, 1, and 30 ppm by injecting O_3 into bags, and then stored at 0°C and 25°C. These O_3 concentrations were selected after preliminary tests using cherries in 1998. Results from the preliminary tests indicated that 30 ppm represented effective pathogen control but with the risk of physiological breakdown, whereas 1 ppm represented decreased susceptibility to physiological breakdown but with the risk of pathogenic attack.

3.3.1.2. Microbial Quality Evaluation

Microbial quality was assessed using fruit washes at 0, 2, 4, 8, 16 and 32 days to detect surface pathogen loads. Ten fruit were aseptically collected into sterile bottles. Peptone water (100 mL, 0.1%) was added to the fruit. The bottles were shaken manually in a 90° arc 25 times and the resulting rinses were serial diluted (10^{-1} to 10^{-6}) with 0.1% peptone water and plated on petrifilms (3M Canada, Inc., London, ON) according to AOAC methods 990.12 and 997.02 (Section E) for aerobic and for yeast and mold counts, respectively (AOAC, 1995). Three replicates were performed.

All experiments were randomized. Statistical analysis was performed using the GLM (General Linear Module) procedure of the Statistical Analysis Software (SAS) (Version 6.11, New Cary, NC). Treatment differences were determined by Duncan's multiple range test ($p=0.05$).

3.3.2. Results, Discussion and Conclusions

Table 3.3.2.1 shows the effect of O_3 concentrations on microbial counts for Pembina and Smoky fruit. Results indicated that, regardless of cultivar, there were no significant differences between the O_3 concentrations for total aerobic, yeast and mold counts. Ozone has been reported to have a number of effects on fruit quality. One effect is as a surface disinfecting agent reducing microbial loads (Barth *et al.*, 1995; Sarig *et al.*, 1996). Another effect is control of the amounts of ethylene in a storage environment which consequently retards respiration and extends shelf-life (Dickson *et al.*, 1992). Ozone has a relatively short half-life (15-50 min). The present work was performed using a single application of O_3. The rationale was that at the concentrations used, a

single application would be sufficient to eliminate surface pathogens. However, it would appear that the O_3 dissipated before its effect was established. Therefore, a continuous application of O_3, perhaps at lower concentrations, may be necessary to determine its effect on shelf-life extension of fresh saskatoon fruit. Additionally, although O_3 may not have significantly affected microbial quality, its effect on fruit physiology, particularly ethylene concentrations in the storage environment, and on quality remain to be investigated. According to Liew and Prange (1994) each commodity responds differently to O_3. Therefore, the effects of O_3 have to be determined for each commodity at its optimal storage temperature. This statement of Liew and Prange (1994) supports the need to pursue further work to determine the efficacy of O_3 treatments on saskatoon fruit.

Storage of fruit at a higher temperature (25°C) significantly increased microbial counts compared to storage at a lower temperature (0°C)(Table 3.3.2.2). Although this result was expected because higher temperatures stimulate the growth of microorganisms, it was surprising that the differences in aerobic, yeast and mold counts between the two temperatures were not greater considering the temperature extremes. These results are preliminary and it would appear that further work is required.

Bag type did not significantly affect microbial counts (Table 3.3.2.3). The rationale for using MA and regular freezer bags was to compare the effectiveness of O_3 treatments in these bags. A second reason for comparing MA and freezer bags was that freezer bags can readily be obtained from a local supermarket whereas MA bags would have to be obtained from a local distributor (Unisource Canada, Inc.). With respect to cost, both bags are comparable ($0.08/bag and $0.09/bag for freezer and MA bags, respectively). This implies that there is no cost advantage of one bag type over the other.

Table 3.3.2.4 shows the overall effect of cultivar on microbial counts. Smoky fruit consistently had lower levels of aerobic, yeast and mold counts compared to Pembina fruit. This result may be important in determining which cultivars are more suitable for the fresh market industry with respect to susceptibility to microbial degradation during storage.

The effect of storage period on microbial load is illustrated in Figure 3.3.2.1. A significant increase in aerobic, yeast and mold counts was observed within 2 days of storage, possibly due to an abundant food supply. Subsequently, a decrease in microbial counts was observed which may be explained by a decrease in nutrient supply and increased competition between the microbes. Yeast and mold colonies were too numerous to count after 4 days of storage at the dilutions used in the study. No significant changes in aerobic counts were found for the remainder of the storage period (4 to 32 days). This implies that changes in microbial quality was generally minimal except for the first two days of storage when sudden growth occurred.

Table 3.3.2.1. Effect of ozone treatments on aerobic, yeast and mold counts for saskatoon fruit (cultivars Pembina and Smoky).

Cultivar	Ozone Conc. (ppm)	Mean Log_{10} Colony Count (cfu/g)		
		Total aerobic	Yeasts	Molds
Pembina	0	4.42*a*	2.01*a*	1.38*a*
	1	4.35*a*	1.92*a*	1.36*a*
	30	4.28*a*	1.77*a*	1.46*a*
Smoky	0	3.89*a*	1.31*a*	0.93*a*
	1	3.86*a*	1.25*a*	0.92*a*
	30	3.90*a*	1.42*a*	0.89*a*

Values represent means of 2 bag types, 2 storage temperatures and 6 storage days. Means in columns for a given cultivar followed by the same letter are not significantly different from each other (p=0.05).

Table 3.3.2.2. Effect of storage temperature on aerobic, yeast and mold counts for saskatoon fruit (cultivars Pembina and Smoky).

Storage temp (°C)	Mean Log_{10} Colony Count (cfu/g)		
	Aerobic	Yeasts	Molds
0	3.51*b*	1.17*b*	0.88*b*
25	5.02*a*	2.27*a*	1.57*a*

Values represent means of 2 cvs, 2 bag types, 3 O_3 concentrations and 6 storage days. Means within columns followed by the same letter are not significantly different from each other (p=0.05).

Table 3.3.2.3. Effect of bag type on aerobic, yeast and mold counts for saskatoon fruit (cultivars Pembina and Smoky).

Bag type	Mean Log_{10} Colony Count (cfu/g)		
	Aerobic	Yeasts	Molds
Freezer	4.13*a*	1.66*a*	1.16*a*
Modified Atmosphere (MA)	4.10*a*	1.56*a*	1.16*a*

Values represent means of 2 cvs, 2 storage temperatures, 3 O_3 concentrations and 6 storage days. Means within columns followed by the same letter are not significantly different from each other (p=0.05).

Table 3.3.2.4. Overall effect of cultivar on aerobic, yeast and mold colonies for saskatoon fruit (cultivars Pembina and Smoky).

Cultivar	Mean Log_{10} Colony Count (cfu/g)		
	Aerobic	Yeasts	Molds
Pembina	4.35*a*	1.90*a*	1.40*a*
Smoky	3.88*b*	1.32*b*	0.91*b*

Values represent means of 2 storage temperatures, 2 bag types, 3 O_3 concentrations and 6 storage days. Means within columns followed by the same letter are not significantly different from each other (p=0.05).

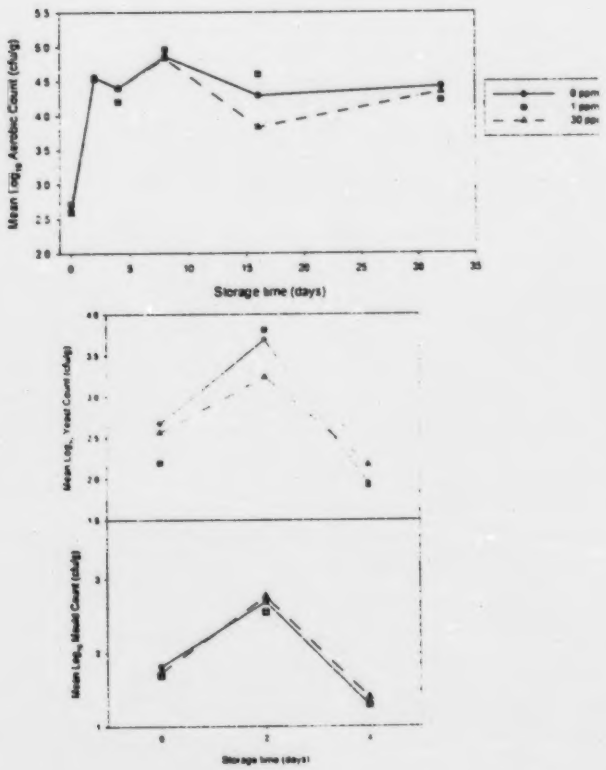

Figure 3.3.2.1. Effect of storage period on aerobic, yeast and mold counts for saskatoon fruit (cultivars Pembina and Smoky). Values represent means of 2 cultivars, 2 bag types and 2 storage temperatures.

3.4. Project Trials - 2000

3.4.1. Materials and Methods

3.4.1.1. Harvest and Post-Harvest Handling of Saskatoon Fruit

Saskatoon fruit of the cultivars Pembina, Smoky and Honeywood were harvested from the University of Saskatchewan plots, Saskatoon, SK. Fruit of the cultivar Thiessen were obtained from a commercial orchard in Waldheim, SK. For practical reasons and due to the differing ripening times of the cultivars, the fruit of Pembina, Thiessen, Smoky and Honeywood were harvested on July 21, 22, 24 and 25, respectively. All fruit were mechanically harvested using a hand-held vibrating comb (BEI Inc., South Haven, MI). After harvest, fruit were transferred into shallow trays (Thunderbird Plastics, Vancouver, BC) which were slotted at the sides and bottom to aid in rapid removal of field heat. Fruit were immediately placed in a cooler. Just prior to harvest, the shelves within the cooler were lined with frozen cryopak ice substitutes (Cryopak Corp, Vancouver, BC). Frozen cryopak maintained the temperature and relative humidity of the cooler at 3 to 5°C and 85 to 90%, respectively, for up to 6 hours. Under these conditions the temperature of fruit dropped by 10°C within 20 minutes. Fruit were held in the cooler for up to 2 hours during transportation and subsequently, for up to 4 hours at 0 to 4°C prior to cleaning.

Fruit cleaning was done using an airdraft fruit cleaner equipped with a sorting table (Hometown Machines, Sundre, AB). Twigs and leaves were aspirated by a blower while fruit exited onto a sorting table for visual inspection to remove defective fruit.

After cleaning, 1.5 kg of fruit were put in shallow trays and placed on a shelf in a ventilated room (Figure 3.4.2.1). The temperature of this room was 0°C. An ozone generator (Crystalair, Delta, BC) was placed directly below the tray containing fruit as shown in Figure 3.4.2.1, and an ozone meter (model #AMC-1108, Armstrong Manufacturing Corp., Nepean, ON) was positioned directly above the tray to measure the ozone concentration. Fruit were exposed to three ozone concentrations, 0 ppm (control), 300 mg/h (measured as 0.4 ppm ozone by the ozone meter), and 600 mg/h (0.8 ppm ozone). Fruit were exposed to ozone for 2 hours.

Following the ozone treatment, 200 g of fruit were weighed into modified atmosphere (MA) bags (Unisource Canada, Inc., Saskatoon, SK), heat-sealed (Deli-Vac machine, Process Plus Inc., Santa Clarita, CA) and immediately stored at 0°C. These MA bags have been shown to extend the shelf-life of fresh saskatoon fruit for a significant period of time. At harvest and subsequently, each week for 4 weeks, samples were taken from cold storage and analyzed for pH, soluble solids content, colour, texture, and microbial quality.

3.4.1.2. Fruit Quality Evaluation

For pH, soluble solids content and colour, 50 g of fruit were placed in sample bags and manually squeezed to produce approximately 15 mls of juice. Care was taken to avoid contaminating the juice with fruit pulp and skin. Soluble solids content was determined using a hand-held refractometer (Fisher Scientific, Model 82322, Tokyo, Japan). Measurements of pH were taken on 5 mls of juice extract using a pH meter (Model 420A, Orion Research Inc., Boston, MA). For colour, 8 mls of juice was placed in a 6 cm-diameter petridish. Colour measurements were obtained using a HunterLab Miniscan XE (Hunter and Associates, Reston, VA). Hunter L, a, and b parameters were recorded (L (lightness) value, 0=black and 100=white; a value, +a=red and -a=green; b value, +b=yellow and -b=blue). Hue angle was obtained from the relationship between the Hunter a and b values using the equation below:

$$\text{Hue Angle} = \tan^{-1}(b/a)$$

Firmness was measured by compressing 10 fruit of relatively the same size with a 10 cm-diameter compression plate. The compression plate was attached to a TA.XT2 texture analyzer (Texture Technologies Corp., Scarsdale, NY) equipped with a 25 kg load cell. Fruit were placed on a circular metal platform (6.5 cm in diameter) with the calyx end facing up, and in a circular pattern approximately 1 cm apart. Crosshead speed of the compression plate during testing was 2.0 mm/s and fruit were compressed to a depth of 7.5 mm (approximately three-quarters of the height). Maximum force required to compress ten fruit was used as an index of firmness.

Yeast and mold populations were assessed from 10 fruit which were aseptically collected into sterile bottles. Peptone water (100 mL, 0.1% w/v) (Merck Chemicals, Darmstadt, Germany) was added to the fruit and the bottles were manually shaken. The resulting rinse were serial diluted (10^{-1} to 10^{-4}) with 0.1% peptone water and plated on petrifilms (3M Canada, Inc., London, ON) according to AOAC methods 997.02 (Section E) (AOAC, 1995).

Ozone treatments were organized as a randomized complete block design with three replicates. Statistical analysis was done using the General Linear Module procedure (proc GLM) of the Statistical Analysis Software (SAS) Program (Version 6.11, New Cary NC). Data on texture were transformed prior to analysis to create normality. Treatment differences were determined by Bonferroni multiple range test ($p=0.05$).

3.4.2. Results and Discussion

3.4.2.1. Molds

Ozone treatment did not affect mold content on the fruit (Table 3.4.2.1). The cultivar Thiessen had a significantly lower mold population on its fruit than the other three cultivars. Mold content on the fruit actually decreased during storage by up to 28% by week 4 (Table

3.4.2.2). This decrease, rather than an increase in mold growth was probably an effect of the modified atmosphere (MA) bags. Storage in these bags may have also masked the effect of ozone treatment on the control of mold.

3.4.2.2. Yeasts

Ozone at the 0.8 ppm rate significantly reduced fruit yeast populations by 20%. The lower concentration of ozone was not effective. A significant cultivar by ozone interaction indicated that the yeast content of Honeywood fruit was significantly lower after exposure to 0.8 ppm ozone compared to that at 0.4 ppm ozone; other cultivars did not differ significantly. Overall, fruit of Pembina and Smoky had a lower yeast content than that of Honeywood and Thiessen. The yeast population increased during storage over that initially contained on the fruit, however, it did not continue to increase with storage but leveled off after the first week. This may have been due to the effect of storage in MA bags, which hampered microbial growth.

3.4.2.3. Total Aerobic Micro-organisms

Total aerobic microorganism (mold + yeast) population on the fruit experienced a significant linear decrease with increasing ozone concentration. Total aerobic count was 14% less on fruit treated with 0.8 ppm ozone than that of the control or the lower rate of ozone. A significant cultivar by ozone interaction indicated that the microorganism content of Honeywood fruit were significantly lower after exposure to 0.8 ppm ozone compared to that at 0.4 ppm ozone; other cultivars did not differ significantly. Overall, fruit of Honeywood had a significantly higher microbial content than that of the other cultivars. The total aerobic count doubled after the first week of storage and then was maintained at approximately this level thereafter in the remaining weeks. The MA bags was likely responsible for restricting the microorganism growth on the fruit during storage.

3.4.2.4. Fruit Texture

The higher the force required to penetrate the fruit, the greater the fruit firmness. Ozone exposure at 0.4 ppm significantly increased fruit firmness over that at the higher rate but not of the control. It is not known why the lower rate of ozone had a greater effect on fruit firmness than the higher rate. A cultivar by ozone interaction indicated that this influence of ozone on fruit firmness occurred only for fruit of Honeywood. Thiessen had the softest fruit and Honeywood and Smoky, the firmest. Fruit firmness increased in a linear fashion with storage time to a maximum of 35%; this increase tended to plateau after the second week of storage.

3.4.2.5. Fruit pH

Overall, ozone exposure tended to increase fruit pH, although that at the higher rate was not significantly different from the control. Fruit treated with ozone at 0.40 ppm increased in pH by 1%. A cultivar by ozone interaction indicated that only fruit pH of the cultivar Smoky was influenced in this manner by ozone treatment. Significant changes in fruit pH due to ozone occurred after two or more weeks of storage. Fruit of Thiessen had the lowest pH and that of Smoky and Honeywood, the highest. Fruit pH increased during storage until week 3 by an average of 7%.

3.4.2.6. Fruit Soluble Solids

Ozone treatments did not affect the soluble solids content of the fruit. Fruit of Thiessen had the lowest and Honeywood, the highest, soluble solid content. Storage only influenced the soluble solid content of the fruit of Thiessen; storage decreased the soluble solid content of the fruit of Thiessen over that found initially.

3.4.2.7. Fruit Colour Intensity

The higher the Hunter L value, the lighter the fruit. Ozone exposure at 0.8 ppm resulted in lighter coloured fruit by 4% over that of the controls. Fruit of the different cultivars each had different L values with fruit of Honeywood being the darkest, and of Thiessen, the lightest. In general, fruit became significantly darker during storage.

3.4.2.8. Fruit Hue

Ozone exposure increased the hue angle of the fruit by an average of 3%. A higher hue angle is indicative of a more reddish hue. Fruit of the cultivars of Honeywood and Thiessen had the lowest hue angle, and Pembina, the highest. Overall, fruit storage tended to result in a slight increase in hue angle to a maximum of 5% in week 2. However, the response during storage tended to be somewhat variable among the different cultivars with the fruit of Honeywood and Thiessen not changing significantly in their hue angle over time.

3.4.3. Conclusions

Ozone exposure was effective in decreasing yeast populations on fruit by 20% at the 0.8 ppm rate. Mold growth was not influenced by ozone treatments in this study although this may have been masked by the effect of storage in MA bags. The total aerobic microbial count experienced a general significant linear decrease in response to increasing ozone rate. Total

microbial growth was 14% less on fruit treated with 0.8 ppm ozone than that of the control or lower ozone rate. Fruit quality parameters were little affected or not at all as a result of exposure to ozone. Fruit pH, L value and hue angle were increased by 1%, 4%, and 3%, respectively, at the higher rate of ozone. The effectiveness of ozone treatments may be more apparent when treating fruit of a higher microbial content, however, both yeast and mold populations on the fruit at the time of harvest were very low. This was also exacerbated by the influence of the MA bags maintaining a low or negative microbial growth during storage. Further research involving ozone treatment of fruit with high microbial populations and storage in regular polyethylene freezer bags are necessary to further discern the efficacy of ozone in reducing yeast and mold content on fruit, and its influence on fruit quality.

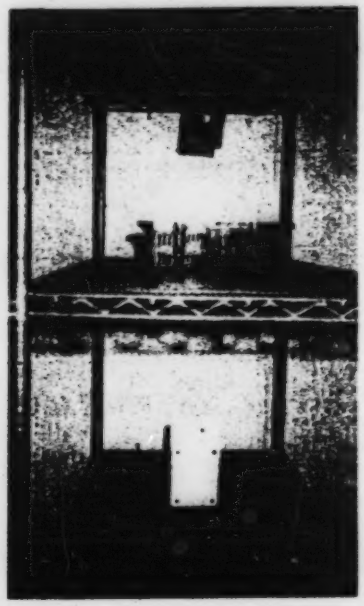

Figure 3.4.2.1. Experimental setup showing an ozone generator on the bottom shelf with the open end directed towards a tray of saskatoon fruit. An ozone meter was hung from the topmost shelf directly above fruit to measure the ozone concentration. All experiments were performed in a ventilated room at 0°C and fruit were exposed to ozone for 2 hours.

Table 3.4.2.1. Mold and yeast populations and quality parameters of saskatoon fruit of 4 cultivars treated with ozone at 3 rates in 2000.

	Mold	Yeast	Total Aerobic	Force	pH	Soluble Solids	Hunter Colour	
	CFU/g[1]	CFU/g	CFU/g	Newtons		°Brix	L	Hue Angle
Ozone Rate								
0 ppm	184	371 b	554	128 ab[2]	4.15 a	16.9	16.9 a	19.0 a
0.4 ppm	192	363 b	554	144 b	4.20 b	16.9	17.4 ab	19.4 b
0.8 ppm	177	298 a	474	124 a	4.16 ab	16.7	17.6 b	19.6 b
S.E.	10.8	16.4	23.8	(0.017)[4]	0.012	0.111	0.158	0.101
Contrasts								
Linear	NS[5]	***	**	NS	*	**	***	***
Quadratic	NS	NS	NS	***	***	NS	NS	NS
Cultivar								
Honeywood	242 b	443 b	682 b	171 c	4.31 c	18.5 c	13.5 a	17.5 a
Pembina	241 b	206 a	447 a	131 b	4.17 b	16.4 b	18.2 c	22.3 c
Smoky	205 b	234 a	439 a	169 c	4.36 c	16.6 b	14.7 b	19.7 b
Thiessen	50 a	494 b	542 a	77 a	3.84 a	15.8 a	22.8 d	17.8 a
S.E.	12.5	18.9	27.4	-0.02	0.013	0.129	0.182	0.116
Mean	184	344	527	-2.65	4.17	16.8	17.3	19.3

1. Coliform forming units per gram of fruit.
2. Means within each main effect within a column with different letters are significantly different by Bonferroni at P=0.05.
3. S.E. = standard error of the mean.
4. Means and standard errors in parentheses are transformed.
5. NS = not significant; *, **, *** significance at 0.05, 0.01 or 0.001, respectively.

Table 3.4.2.2. Mold and yeast populations and quality parameters of saskatoon fruit of 4 cultivars treated with ozone at 3 rates after 0 to 4 weeks of storage at 0°C in 2000.

	Mold	Yeast	Total Aerobic	Force	pH	Soluble Solids	Hunter Colour	
	CFU/g[1]	CFU/g	CFU/g	Newtons		°Brix	L	Hue Angle
Week 0	218 b[2]	159 a	371 a	109 a	4.00 a	17	16.7 d	18.7 a
Week 1	209 ab	347 b	556 b	119 ab	4.10 b	17	17.8 c	19.4 ab
Week 2	171 ab	419 b	590 b	140 b	4.18 c	16.8	17.5 c	19.7 b
Week 3	168 ab	421 b	587 b	148 b	4.28 d	16.6	16.7 b	19.4 ab
Week 4	156 a	375 b	533 b	146 b	4.28 d	16.8	15.9 a	19.5 ab
Mean	18411	34424.7	527	(2.65)[4]	4.17	16.8	17.3	19.3
S.E.[3]				(0.022)	0.005	0.090	0.128	0.170
Contrasts								
Linear	***[5]	***	***	***	***	***	***	***
Quadratic	NS	***	***	*	***	NS	NS	***
Cubic	NS	NS	NS	NS	***	*	NS	*

1. Coliform forming units per gram of fruit.
2. Means within each main effect within a column with different letters are significantly different by Bonferonni at P=0.05.
3. S.E. = standard error of the mean.
4. Means and standard errors in parentheses are transformed.
5. NS = not significant; *, **, *** significance at 0.05, 0.01 or 0.001, respectively.

3.5. Project Trials - 2001

3.5.1. Materials and Methods

3.5.1.1. Harvest and Post-Harvest Handling of Saskatoon Fruit

In the 2000 season there may have been an inadequate micorooganism population on the fruit at the onset to clearly ascertain the effect of ozone. Therefore, additional pre-treatments of the fruit prior to ozone exposure were included in the study to ensure the presence of microorganisms on the fruit. Spore suspensions were sprayed onto the fruit to ensure the presence of microorganisms. Two controls were also included, one in which fruit were sprayed with water, and an unsprayed control. Saskatoon fruit of the cultivars Smoky and Honeywood were harvested from the University of Saskatchewan plots, Saskatoon, SK. Fruit of the cultivar Thiessen were obtained from a commercial orchard in Waldheim, SK. For practical reasons and

due to differing ripening times of the cultivars, the fruit of Thiessen, Honeywood and Smoky were harvested on July 14, 17 and 19, respectively. All fruit but Thiessen were mechanically harvested using a hand-held vibrating comb (BEI Inc., South Haven, MI). Fruit of the cultivar Thiessen were hand-picked. After harvest, fruit were immediately placed in a portable cooler. Just prior to harvest, the shelves within the cooler were lined with frozen cryopak ice substitutes (Cryopak Corp, Vancouver, BC). Frozen cryopak can maintain the temperature and relative humidity of the cooler at 3 to5°C and 85 to 90%, respectively, for up to 6 hours. Fruit were held in the cooler for up to 2 hours during transportation. Once the fruit reached their destination, they were placed in a 5°C cooler prior to cleaning and sorting.

Fruit cleaning was done using an airdraft fruit cleaner equipped with a sorting table (Hometown Machines, Sundre, AB). Twigs and leaves were aspirated by a blower while fruit exited onto a sorting table for visual inspection to remove defective fruit.

After cleaning, 900 g of fruit were put in shallow, slotted trays (Thunderbird Plastics, Vancouver, BC). This fruit covered the bottom of the tray in one layer. The fruit were sprayed with 20 mls of a spore suspension (a mixture of both *Botrytis* and *Alternaria alternata* spores each at a concentration of approximately 10^4 spores/ml) in distilled water, 20 mls of distilled water, or left unsprayed. The fruit were left to dry in the trays at room temperature for 2 hours and then half the trays were placed in a 0°C cooler and exposed to ozone for one hour. The other half of the fruit were placed in another 0°C cooler for one hour with no ozone. Ozone exposure was accomplished by two ozone generators (Crystalair, Delta, BC) each set at 300 mg/h (the equivalent of 0.8 ppm) placed underneath the trays. Following the ozone treatment, 200 g of fruit were weighed into polyethylene freezer bags (Superstore, Saskatoon, SK), heat-sealed (Deli-Vac machine, Process Plus Inc., Santa Clarita, CA) and stored at 0°C. At harvest and each following week for 3 weeks, samples were taken from cold storage and analyzed for pH, soluble solids content, colour, texture, titratable acidity and microbial content.

3.5.1.2. Fruit Quality Evaluation

For the assessment of pH, soluble solids content and colour, 50 g of fruit were placed in sample bags and manually squeezed to produce approximately 15 mls of juice. Care was taken to avoid contaminating the juice with fruit pulp and skin. Soluble solids content was determined using a hand-held refractometer (Fisher Scientific, Model 82322, Tokyo, Japan). Measurements of pH were taken from a 5 ml juice extract using a pH meter (Model 420A, Orion Research Inc., Boston, MA). Titratable acidity was analyzed by the electrode method (AOAC, 1995). Juice was extracted from approximately 50 g of frozen fruit by boiling for 1 hour in 200 mls of deionized water. The pulp and juice were centrifuged at 8500 rpm for 20 minutes. The supernatant was titrated to a pH of 8.1 with 0.1 N NaOH, and expressed as % malic acid. Colour measurements were obtained using a Hunter Miniscan XE (Hunter and Associates, Reston, VA) from 8 mls of juice placed in a 6 cm diameter pertridish. Hunter L, a, and b parameters were recorded (L (lightness) value, 0=black and 100=white; a value, +a=red and -a=green; b value, +b=yellow and -b=blue). Hue angle was calculated from the Hunter a and b values using the equation below:

Hue Angle = $\tan^{-1}(b/a)$

Firmness was measured by compressing 10 fruit of relatively the same size with a 10 cm-diameter compression plate. The compression plate was attached to a TA.XT2 texture analyzer (Texture Technologies Corp., Scarsdale, NY) equipped with a 25 kg load cell. Fruit were placed on a circular metal platform (6.5 cm in diameter) with the calyx end facing up, and in a circular pattern approximately 1 cm apart. Crosshead speed of the compression plate during testing was 2 mm/s and fruit were compressed to a depth of 7.5 mm (approximately three-quarters of the height). Maximum force required to compress 10 fruit was used as an index of firmness.

Assessment of yeast and mold populations were obtained from a sample of 10 fruit. The fruit were aseptically collected into sterile bottles, peptone water (100 ml, 0.1% w/v) (Merck Chemicals, Darmstadt, Germany) was added to the fruit and the bottles were manually shaken. The resulting rinse were serially diluted (10^{-1} to 10^{-4}) with 0.1% peptone water and plated on petrifilms (3M Canada, Inc., London, ON) according to AOAC methods 997.02 (Section E) (AOAC, 1995).

Treatments were organized as a randomized complete block design with three replicates. Statistical analysis was done using the General Linear Module procedure (proc GLM) of the Statistical Analysis Software (SAS) Program (Version 6.11, New Cary NC). Data on mold and total aerobic content, fruit texture, pH, titratable acidity and hue angle were transformed prior to analysis to create normality. Treatment differences were determined by Bonferroni multiple range test ($p=0.05$).

3.5.2. Results and Discussion

3.5.2.1. Molds

Ozone decreased mold content of the fruit by 20% overall (Table 3.5.2.1). A week by ozone interaction indicated that ozone caused a significant decrease of mold in week 2; during this week mold populations were 44% lower for ozone treated fruit. In general, Thiessen had the lowest and Honeywood the greatest mold content on the fruit. Pre-treatment sprays on the fruit affected mold populations. Fruit sprayed with water had more mold growth than those unsprayed or sprayed with micro-organisms overall. This trend differed over weeks and for each cultivar. Spray treatments did not affect mold populations of Thiessen. Smoky was only affected by the spray treatment initially in which the fruit treated with spores had less mold growth than those of the water and dry controls. The cultivar Honeywood was most affected by the spray treatments. Initially mold growth was greater on dry fruit than on those treated with spores, however, by week 2 and 3 dry fruit of Honeywood had less mold than those sprayed with water or microorganisms. It appears that for the cultivars Honeywood and Smoky, the sprays with microorganisms may have had an initial antagonistic effect against the growth of other mold spores that were already present on the fruit. However, the additional moisture on the fruit that were sprayed with either water or microorganisms (fruit did not dry completely before

packaging) contributed to mold growth during storage resulting in greater mold content on the fruit that were sprayed than on the dry fruit. In general, fruit sprayed with spores did not increase mold growth and actually decreased it initially for two of the cultivars; it appears rather that the excess moisture alone contributed to greater mold content on the fruit during storage. Mold growth increased significantly with each subsequent week of storage reaching a level 180 times greater by week 3 (Table 3.5.2.2).

3.5.2.2. Yeasts

The ozone treatment decreased yeast populations on fruit by 10% overall, however this decrease was not significant. In general, the fruit of Thiessen had the lowest and Smoky the highest yeast content. Differences among cultivars existed only during the last two weeks of storage. Spray pre-treatments differed significantly in yeast content. Dry fruit had less yeast growth overall than those sprayed with water or spores. This was only significantly different for Honeywood and Smoky during the last two weeks of storage; fruit of Honeywood and Smoky in week 2 had significantly less yeast on dry fruit than on those sprayed with spore suspensions, and fruit of Smoky in week 3 had less yeast on dry fruit than on those sprayed with both water and spores. Because spray pre-treatments only contained mold spores, any increase in yeast growth on fruit sprayed with the microorganism suspension would be due to the influence of the added moisture alone. Yeast populations on the fruit significantly increased with each subsequent week in storage reaching a level 129 times greater by week 3.

3.5.2.3. Total Aerobic Micro-organisms

Ozone exposure significantly reduced the total aerobic microbial (mold + yeast) population on saskatoon fruit by 14%. Fruit of Thiessen had a significantly lower microbial content than that of Smoky and Honeywood. Overall, dry fruit also had a lower microbial content than those sprayed with water or microorganisms prior to ozone exposure. This was particularly apparent during weeks 2 and 3; spray treatments did not cause significant differences in microbial growth at the onset which would be expected. Fruit microorganism populations increased with each subsequent week in storage reaching a level 134 times greater in week 3 than at the time of harvest.

3.5.2.4. Fruit Texture

Texture as measured by the force required to penetrate through the fruit is expressed in newtons. Ozone treatment had no affect on the texture of the fruit. Fruit of Thiessen was the least firm and fruit of Smoky the most firm of the cultivars. Fruit of Honeywood sprayed with water did not increase as much in firmness during storage compared to those left dry or sprayed with spores during week 2 and 3. Spray treatments had no effect on the fruit of Thiessen. Fruit of Honeywood sprayed with water increased significantly less in firmness than those left dry or

sprayed with spores. Fruit of Smoky left dry were less firm than those sprayed with spores. Spray treatments did not affect firmness initially but by week 2 and 3, fruit treated with microorganisms or left dry were firmer than those sprayed with water. Fruit became significantly firmer during storage increasing in firmness by 69% in week 3. An increase in saskatoon fruit firmness during storage has been observed in other studies and is probably associated with water loss and/or the physicochemical breakdown of the fruit during storage.

3.5.2.6. Fruit pH

Exposure to ozone significantly decreased the pH of the fruit by 0.7%, while storage significantly increased fruit pH by a range of 2.3% after one week to an increase of 11% after three weeks. Cultivars differed in pH with the fruit of Thiessen having the lowest pH and Smoky, the highest. Spray treatments had an effect on the pH of the fruit of Honeywood; fruit sprayed with water had a significantly lower pH than those left dry or sprayed with spores.

3.5.2.7. Fruit Soluble Solids

Soluble solids content of the fruit was not affected by ozone treatment. In general, the fruit of Thiessen had the lowest soluble solids content and Honeywood the highest. Spray treatments had an effect on soluble solid content, however, this effect differed for each cultivar and no consistent pattern existed. Soluble solids content of the fruit significantly decreased during weeks 2 and 3 of storage by 5%.

3.5.2.8. Fruit Titratable Acidity

Titratable acidity of the fruit was not influenced by the ozone treatment. Cultivars differed in their titratable acidity with Thiessen being the most acidic and Smoky, the least. Overall, fruit sprayed with water before storage were 8% more acidic than the dry fruit. However, by week 2 and 3 of storage no differences were found among the spray treatments. A cultivar by spray interaction indicated that the titratable acidity of dry fruit and those sprayed with microorganisms of the cultivar Honeywood were significantly lower than for the fruit sprayed with water; titratable acidity of Thiessen and Smoky were not affected by the spray treatments. Titratable acidity of the fruit decreased throughout the storage period becoming 45% less acidic by week 3.

3.5.2.9. Fruit Colour Intensity

Ozone did not have an effect on the Hunter L value of the fruit. Low Hunter L values are indicative of darker fruit. The cultivar Honeywood had the darkest fruit and Thiessen the lightest. Dry fruit were the darkest and those sprayed with water, the lightest in colour. Fruit tended to

become significantly darker during storage.

3.5.2.10. Fruit Hue

Ozone treatment of the fruit significantly increased the hue angle by 2%. A higher hue angle is indicative of a more reddish hue. Overall, fruit of Smoky were the most reddish and that of Honeywood, the least. This, however, may be a factor of the degree of fruit maturity at the time of harvest and not an actual cultivar characteristic. Spray treatments significantly influenced hue angle; overall, fruit sprayed with water had a 6% and 5% increase in hue angle compared to the dry fruit, and those sprayed with spores, respectively. Spray treatments influenced hue angle differently for the different cultivars; Honeywood was not affected while fruit of Thiessen sprayed with spores had a lower hue angle than the water and dry controls. Dry fruit of Smoky had a lower hue angle than those fruit sprayed with spores or water. The cultivars differed in their change in hue angle during storage. The hue angle of the fruit of Thiessen increased by 59% during storage and Honeywood by only 12%; Smoky was intermediate between the two. Initially Thiessen had the lowest hue angle of the three cultivars, but the highest by week 3 of storage. Storage significantly increased hue angle of the fruit each week reaching a 29% increase by week 3.

3.5.3. Conclusions

Ozone was effective in reducing the mold population of saskatoon fruit by 20%. Yeast populations were reduced due to ozone by 10% although this reduction was not significant. Total fruit microbial content was significantly reduced by 14% due to ozone treatment. Fruit quality largely was not affected by ozone treatment. Fruit pH was reduced by 1% and hue angle was increased by 2% due to ozone exposure otherwise no significant change occurred in fruit quality. This research indicates that ozone may be an effective treatment to improve fresh fruit storage without compromising fruit quality.

Table 3.5.2.1. Mold and yeast populations and quality parameters of saskatoon fruit treated with ozone and inoculated with microorganisms prior to packaging and storage at 0°C for 3 weeks in 2001.

	Mold	Yeast	Total Aerobic	Texture	pH	Soluble Solids	Titratable Acidity	Hunter Colour	
	CFU/g[1]	CFU/g	CFU/g	Newtons		°Brix	%	L	Hue Angle
Ozone Treatment									
None	10071 a[2]	60448	49822 b	56.1	4.19 a	14.3	0.26	17.5	19.5 a
Ozone	8020 b	54286	42741 a	54.4	4.16 b	14.4	0.26	17.8	19.9 b
S.E.[3]	(0.226)[4]	2532	(4.01)	(0.057)	(0.001)	0.061	(0.0027)	0.124	(0.014)
Cultivar									
Honeywood	25431 c	57515 b	79815 b	59.4 b	4.30 b	16.2 c	0.24 b	12.4 a	18.1 a
Smoky	14934 b	109201 c	86512 b	70.0 c	4.38 c	14.1 b	0.18 a	15.0 b	21.3 c
Thiessen	730 a	5385 a	4662 a	38.8 a	3.85 a	12.7 a	0.37 c	25.5 c	19.8 b
S.E.	(0.277)	3101	(4.91)	(0.069)	(0.001)	0.075	(0.0033)	0.151	(0.017)
Spray Treatment									
Dry Control	7213 a	42925 a	33593 a	56.2	4.23 c	14.5	0.25 a	17.0 a	19.4 a
Water Control	11229 b	62313 b	52864 b	50.6 a	4.12 a	14.3	0.27 b	18.2 c	20.2 c
Microorganisms	8882 a	66863 b	53692 b	59.1 b	4.17 b	14.3	0.26 ab	17.7 b	19.5 b
S.E.	(0.277)	3101	(4.91)	(0.069)	(0.001)	0.075	(0.0033)	0.151	(0.017)
Mean	(15.36)[4]	57367	(215)	(7.43)	(1.54)	14.4	(0.508)	17.6	(4.44)

1. Coliform forming units per gram of fruit.
2. Means within each main effect within a column with different letters are significantly different by Bonferroni at P=0.05.
3. S.E. = standard error of the mean.
4. Means and standard errors in parentheses are transformed.

Table 3.5.2.2. The effect of storage at 0°C for three weeks on the mold and yeast populations and quality parameters of saskatoon fruit in 2001.

	Mold	Yeast	Total Aerobic	Texture	pH	Soluble Solids	Titratable Acidity	Hunter Colour	
	CFU/g[1]	CFU/g	CFU/g	Newtons		°Brix	%	L	Hue Angle
Week 0	341 a[2]	1161a	1495 a	38.2 a	3.97 a	14.8 b	0.38 d	18.0 bc	17.3 a
Week 1	3223 b	10760 b	12869 b	59.1 b	4.06 b	14.5 b	0.28 c	18.6 c	19.1 b
Week 2	12946 c	67496 c	67795 c	61.2 b	4.25 c	14.1 a	0.22 b	17.4 ab	20.3 c
Week 3	61025 d	150051 d	200187 d	64.7 b	4.42 d	14.1 a	0.17 a	16.6 a	22.3 d
Mean	(15.36)[4]	573671012	(215)	(7.43)	(1.535)	14.36	(0.508)	17.6	(4.44)
S.E.[3]	(0.120)		(1.24)	(0.079)	(0.0003)	0.057	(0.0021)	0.171	(0.017)
Contrast									
Linear	***[5]	***	***	***	***	***	***	***	***
Quadratic	***	***	***	***	***	*	***	***	NS
Cubic	**	NS	NS	***	***	*	NS	***	**

1. Coliform forming units per gram of fruit.
2. Means within each main effect within a column with different letters are significantly different by Bonferroni at P=0.05.
3. S.E. = standard error of the mean.
4. Means and standard errors in parentheses are transformed.
5. NS = not significant; *, **, *** significance at 0.05, 0.01 or 0.001, respectively.

3.6. General Summary and Conclusions

Exposure to ozone at the rate of 0.8 ppm for one to two hours prior to the packaging of saskatoon fruit was effective in reducing microbial populations on the fruit by 14% in both years of the study. Ozone treatment reduced yeast content by 20% in 2000 and 10% in 2001 although this reduction was only significant in the first year. Mold content on the fruit was significantly reduced by 20% in 2001 by ozone exposure. The modified atmosphere (MA) bags used in 2000 influenced yeast and mold growth during storage by hampering their growth. These bags probably masked the effect of the ozone treatments that year. Microbial populations of mold in the MA bags actually decreased instead of increased during storage. Microbial populations of the fruit at harvest were quite low in 2000 (average of 175 yeast cfu/g and 239 mold cfu/g) compared to the cultivars of Smoky and Honeywood in 2001 (average of 1990 yeast cfu/g and 2868 mold cfu/g). However, the cultivar Thiessen in 2001 had no measurable yeast and mold at harvest but this increased to 13282 yeast cfu/g and 6540 mold cfu/g by week 3 in storage. In comparison, in 2000, fruit in MA bags only increased to an average of 396 yeast cfu/g and 169 mold cfu/g by the end of 4 weeks of storage. Thus, it is evident that the MA bags and not the low initial microbial populations contributed to the apparent lack of response of fruit mold content to ozone in 2000.

Fruit quality was little or not affected by ozone treatment. In 2000, fruit pH, Hunter L value and hue angle were increased due to ozone exposure by 1%, 4% and 3%, respectively. In 2001, fruit pH was reduced by 1% and hue angle was increased by 2% after ozone treatment otherwise no significant changed occurred in fruit quality. It is not known why pH was increased during the first year of the experiment and decreased in the second although this may be associated with the use of MA bags in 2000 and polyethylene bags in 2001. Overall, these small changes to fruit pH and colour (slightly lighter and more reddish fruit), although statistically significant, do not represent a reduction in fruit quality for all practical purposes.

Further research aimed at increasing the effectiveness of ozone treatment and examining the reliability of its use would be warranted. The economic feasibility of its use also needs to be investigated.

3.7. References

AOAC 1995. Official Methods of Analysis. 7th edition. AOAC International, Gaithersburg, MD.

Barth, M.M., Zhou, C., Mercier, J., and Payne, F.A. 1995. Ozone storage effects on anthocyanin content and fungal growth in blackberries. J. Food Sci. 60(6):1286-1288.

Dickson, R.G., Kays, S.J., Law, S.E., and Eiteman, M.A. 1992. Abatement of ethylene by ozone treatments in controlled atmosphere storage of fruits and vegetables. Paper No. 92-6571, Am-Soc-Agric-Eng. St. Joseph, MI.

Liew, C.L. and Prange, R.K. 1994. Effect of ozone and storage temperature on postharvest

diseases and physiology of carrots (*Daucus carota* L.). J. Amer. Soc. Hort. Sci. 119(3):563-567.

Garrett, E. and McInerney, M. 1997. Voluntary Food Safety Guidelines For Fresh Produce. A publication of the International Fresh-cut Produce Association and Western Growers Association.

Sarig, P., Zahavi, T., Zutkhi, Y., Yannai, S., Lisker, N., and Ben-Arie, R. 1996. Ozone control of post-harvest decay of table grapes caused by *Rhizopus stolonifer*. Physiol. Mol. Plant Pathol. 46(6):403-415.

4. Personnel

R. St-Pierre - 5%
A. Zatylny - 20%
S. Agblor - 20%
W. Pomedli - 20%
R. Spencer - 5%

5. Equipment

None.

6. Project Developed Materials

St-Pierre, R.G. 2001. Effects of Ozone Treatments on Shelf-life Extension, Physiology and Quality of Fresh Saskatoon Berries. Progress Report - February 15, for Saskatchewan Agriculture and Food, Agriculture Development Fund.

R.G. St-Pierre, 2000. Effects of Ozone Treatments on Shelf-life Extension, Physiology and Quality of Fresh Saskatoon Berries. Progress Report - April 1, for Saskatchewan Agriculture and Food, Agriculture Development Fund.

St-Pierre, R.G. 1999. Effects of ozone treatments on shelf-life extension, physiology and quality of fresh saskatoon berries. Progress Report - October 1, for Saskatchewan Agriculture and Food, Agriculture Development Fund.

7. Project Photos

Inserted in report.

8. Acknowledgement

St-Pierre, R.G. 2001. Effects of Ozone Treatments on Shelf-life Extension, Physiology and Quality of Fresh Saskatoon Berries. Progress Report - February 15, for Saskatchewan Agriculture and Food, Agriculture Development Fund.

R.G. St-Pierre, 2000. Effects of Ozone Treatments on Shelf-life Extension, Physiology and Quality of Fresh Saskatoon Berries. Progress Report - April 1, for Saskatchewan Agriculture and Food, Agriculture Development Fund.

St-Pierre, R.G. 1999. Effects of ozone treatments on shelf-life extension, physiology and quality of fresh saskatoon berries. Progress Report - October 1, for Saskatchewan Agriculture and Food, Agriculture Development Fund.

9. Expense Statement

Sent under separate cover by Research Accounts, University of Saskatchewan.